Defensin

Week 8

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Digestion of pSB1C3-Def plasmids with PCR analysis

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
Gel electrophoresis

Modifications and comments to protocols

3A Assembly: total volume 20 μl (digestion mix)
3A Assembly: 1 μg of DNA in digestion mix

Experimental Set Up

Table 1: Concentration of samples used for digestion.

Sample	Concentration [ng/μl]
Nuc 1	88.2
Nuc 4	74.6

Table 2: Volumes of digestion mix components for both samples.

Component	Nuc 1 [μl]	Nuc 4 [μl]
DNA template	5.76	6.7
10x SuRE/Cut Buffer H	1	1
XbaI	0.5	0.5
PstI	0.5	0.5
ddH_2O	2.33	1.3

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (1)$$

Results and Conclusions

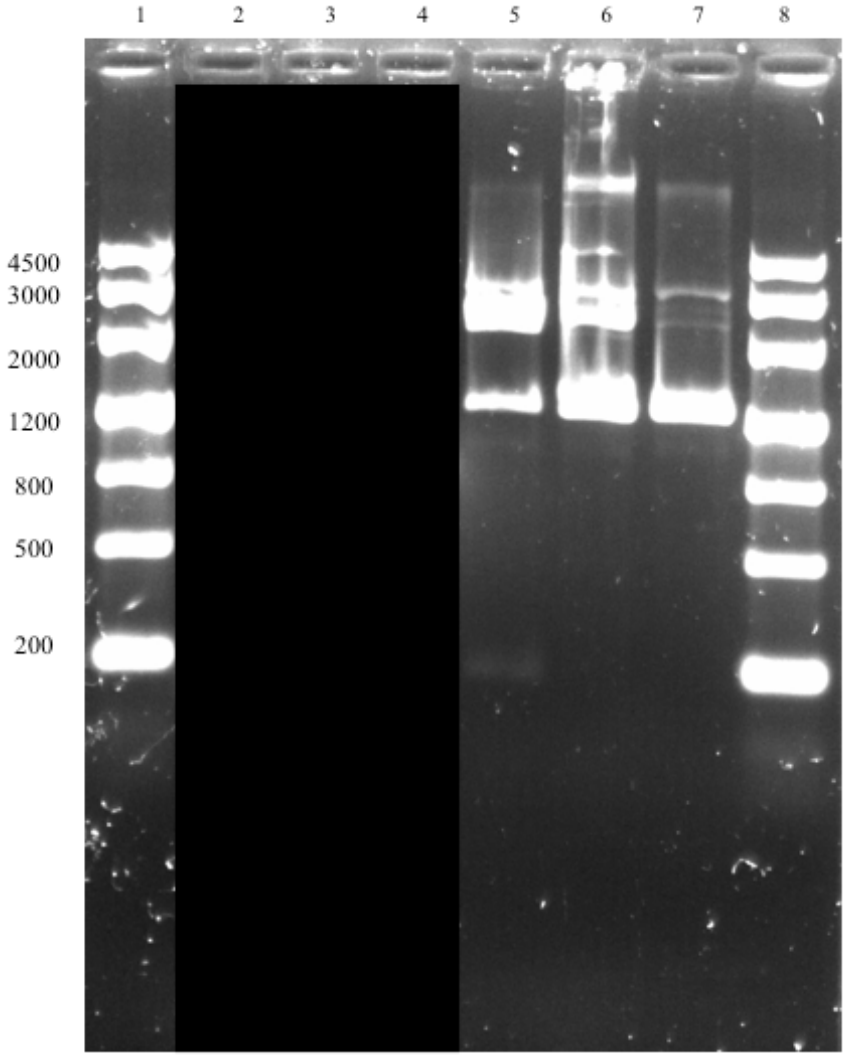

Figure 1: Result of the gel electrophoresis of PCR product, with 1 % agarose at 100 V. (1) DNA Ladder (5) Def 1 (6) Def 4 (7) Negative control (8) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

No bands at expected size of 162 bp - in fact the gels does not seems to show anything corresponding even to undigested PCR product and the negative control was highly contaminated. The experiment was re-done, starting with the PCR amplification.

2 PCR amplification of extracted pSB1C3-Def plasmids

Responsible

Oscar He and Ellinor Lindholm

Protocols used

PCR Amplification (Q5)
Gel electrophoresis

Modifications and comments to protocols

PCR Amplification (Q5) primers:	VR (reverse) VF2 (forward)

Results and Conclusions

Table 3: Concentration of PCR product measured with NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Def HQ	392.6

3 Digestion of pSB1C3-Def PCR amplification product

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly

Experimental Set Up

3A Assembly was set up the usual way and no modifications to the protocol were made.

Sample Calculation

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \tag{2}$$

Results and Conclusions

Digestion product not analyzed.

Discussion and Troubleshooting

The digestion product was used for ligation without analysis.

4 3A assembly (ligation) of pSB1C3-T7-Def

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
PCR Amplification (Q5)
Gel electrophoresis

Modifications and comments to protocols

	Amount of vector per reaction	50.0 ng
3A Assembly:	Ratio vector:insert	1:3
	Incubation	14 h at 16 °C
PCR Amplification (Q5) primers:		VF2_R (reverse)
		VF2_F (forward)

Experimental Set Up

Table 4: Concentration and size of the parts used for the ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	2.3	2070
T7	6.6	32
Def HQ	4.0	162

Table 5: Volumes of each component used in the ligation mix.

Component	Volume [μl]
pSB1C3	21.74
T7	0.52
Def HQ	2.76
T4 DNA Ligase	0.5
10x T4 DNA Ligase Buffer	3
ddH_2O	1.48

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1C3, T7 and defensin

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \quad (3)$$

$$Volume\ of\ insert = Ratio \cdot \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{4}$$

Results and Conclusions

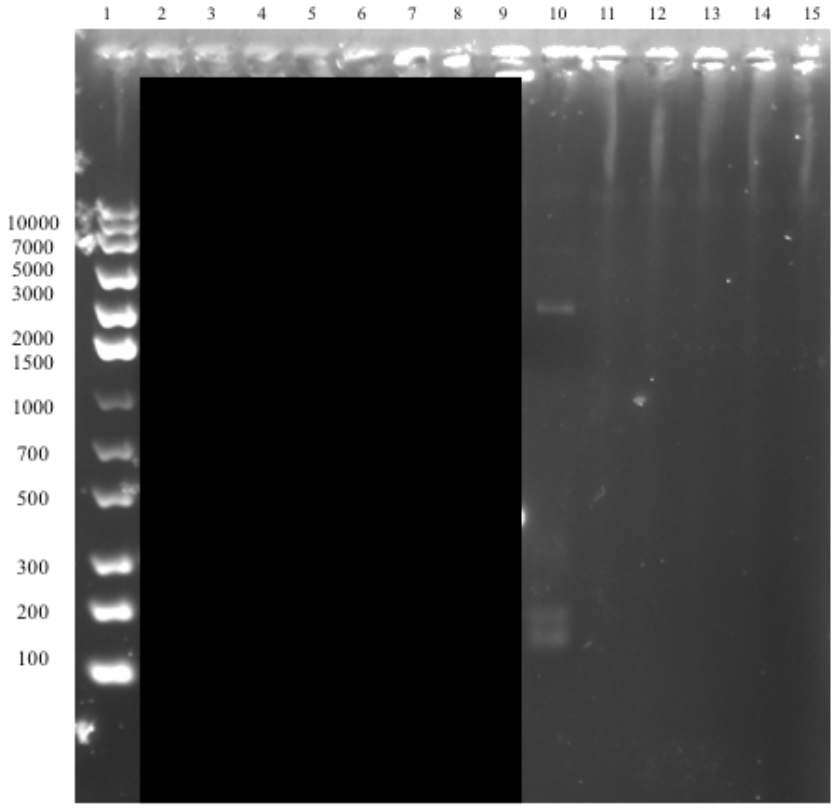

Figure 2: Result of the gel electrophoresis of PCR product, with 1 % agarose at 100 V. (1) DNA Ladder (10) Def ligation. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

Discussion and Troubleshooting

The gel showed a faint band at the expected size at 2264 bp. Hence, the digestion product was further transformed.

5 Transformation of pSB1C3-T7-Def in Top10 and BL21(DE3)

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Transformation
Colony picking

Experimental Set Up

Table 6: Volumes of liquid culture for each picked colony.

Sample	Volume [μl]
Def Top10 1	5
Def Top10 2	5
Def Top10 3	5

Results and Conclusions

Only transformation into Top10 was successful.

Discussion and Troubleshooting

Since there were only transformed Top10 cells, only those were inoculated in liquid media.

6 Extraction of pSB1C3-T7-Def from transformed Top10 cells with PCR analysis

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Plasmid Extraction
PCR Amplification (Q5)
Gel electrophoresis

Modifications and comments to protocols

Plasmid Extraction - Step 1: centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
Plasmid Extraction - Step 10: DNA elution using EB Buffer

Experimental Set Up

Table 7: Volumes of the liquid cultures used for plasmid extraction.

Sample	Volume [μl]
Def Top10 1	1.5
Def Top10 2	1.5
Def Top10 3	1.5

Table 8: Calculated concentration of sample plasmid in the PCR mix.

Sample	Concentration [ng/μl]
Def Top10 1	1.322
Def Top10 2	1.952
Def Top10 3	1.542

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \tag{5}$$

Results and Conclusions

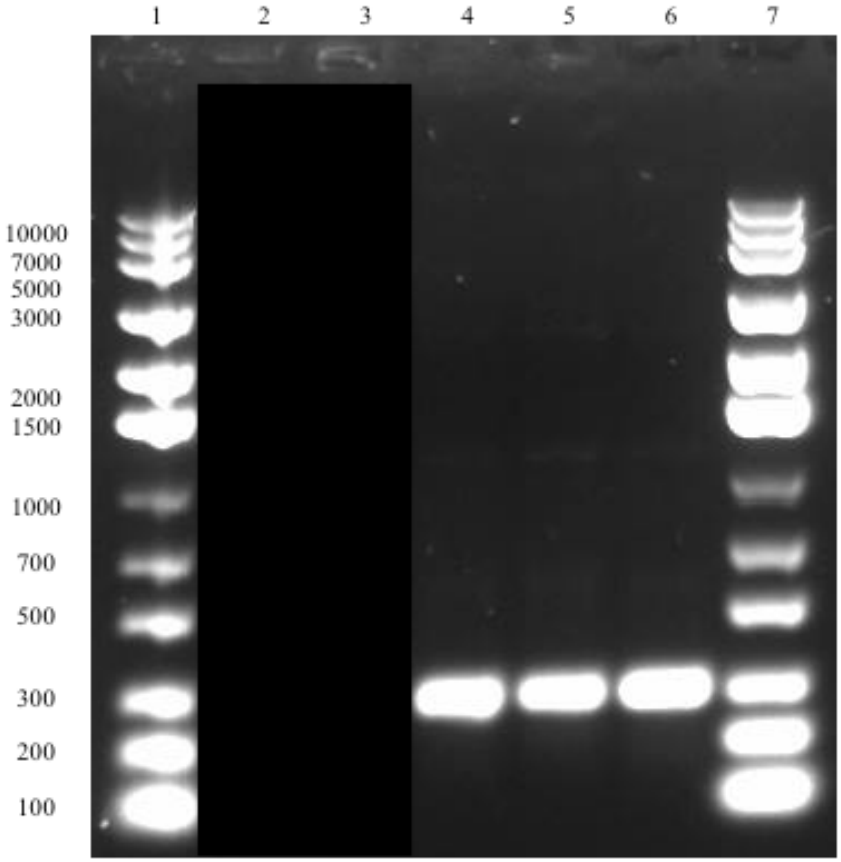

Figure 3: Result of the gel electrophoresis of PCR product, with 1 % agarose at 100 V. (1) DNA Ladder (4) Def Top10 1 (5) Def Top10 2 (6) Def Top10 3 (7) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

Table 9: Concentrations of the extracted plasmid samples.

Sample	Concentration [ng/μl]
Def Top10 1	33.05
Def Top10 2	48.8
Def Top10 3	38.55

Table 10: Concentrations of PCR product of the extracted plasmid samples.

Sample	Concentration [ng/μl]
Def Top10 1	264.2
Def Top10 2	266.85
Def Top10 3	263.0

Discussion and Troubleshooting

The PCR products were all three sent for sequencing to check what the samples actually contains. This since it was not the first time this particular result had been obtained. However, a mistake was made and two primers were added to the sequencing mixture, unfortunately resulting in a nonsense sequencing result.

7 3A assembly (ligation) of pSB1C3-T7-Def

Responsible

Oscar He and Ellinor Lindholm

Protocols used

3A Assembly
Gel electrophoresis

Modifications and comments to protocols

3A Assembly (ligation):	Amount of vector per reaction	34.5 ng
	Ratio vector:insert	1:10
	Incubation	14 h at 16 °C

Experimental Set Up

Table 11: Concentration and size of the parts used for the ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	11.5	2070
T7	71.8	32
Def	38.7	162

Table 12: Volumes of each component used in the ligation mix.

Component	Volume [μl]
pSB1C3	3
T7	7
Def	7
T4 DNA Ligase	1
10x T4 DNA Ligase Buffer	2
ddH_2O	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1C3, T7 and defensin

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{6}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{7}$$

Results and Conclusions

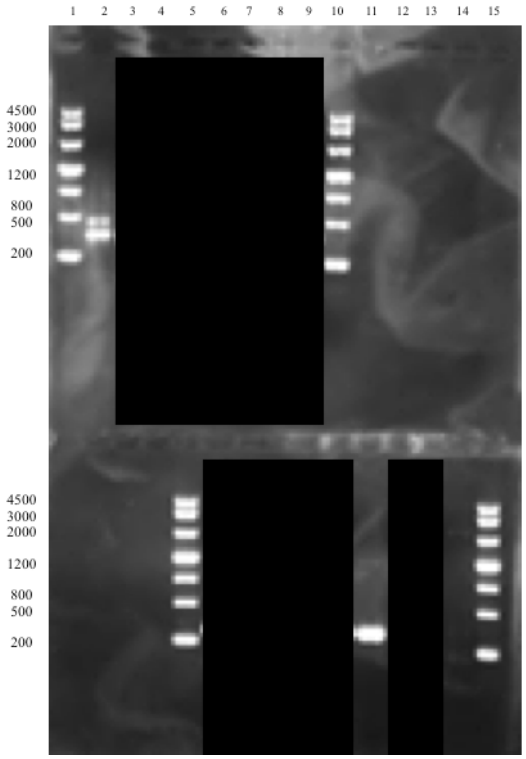

Figure 4: Result of the gel electrophoresis, with 1 % agarose at 100 V. (1) DNA Ladder (2) Def ligation PCR product (10) DNA Ladder (5) DNA Ladder (11) Def Top10 (14) Def ligation product (15) DNA Ladder. The wells hidden by the black rectangle correspond to samples unrelated to Defensin that were run on the same gel.

Discussion and Troubleshooting

The vector:T7 ratio was increased to the highest possible (with regards to the amount of T7 digestion product available). The analysis of the ligation product was performed later. Hence, it was used for further experiments prior to confirming a successful ligation product.